Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-16, 18-54 and 57-62 are pending in the application, with claim 1 being the independent claim. Claim 17 is sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 58-62 are sought to be added. Claims 1-13, 15, 16, 18-34, 36–43, 46-49, 51, 52 and 54 are sought to be amended. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Informalities

The disclosure has been objected to because at page 22 because "no Example 6 is described." (Office Action, page 2, line 14). This is an obvious typographical error. The specification has been amended at pages 22 and 23 to correct the headings to read, "Example 6," "Example 7" and "Example 8," respectively. Thus, the Examiner's objection has been accommodated.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-27 and 32-54 were rejected under 35 U.S.C. § 112, first paragraph.

The Examiner has raised a number of "enablement" objections in view of the terms "derivative" and "fragment" as they appear in the claims.

The term "derivative" has been deleted from the claims in order to expedite prosecution. The Examiner's objection to this term has been accommodated.

Regarding the term "fragment," this term is qualified in the claims as a component of the L-chain of a clostridial neurotoxin which includes the active proteolytic enzyme Domain of the L-chain.

An applicant is not limited to the confines of the specification to provide the necessary information to enable the invention; there are two other sources of enabling information. *In re Howarth*, 654 F.2d 103, 105-6, 210 U.S.P.Q. 689, 692 (C.C.P.A. 1981). An applicant need not supply information that is well known in the art. *Howarth*, 654 F.2d at 105-6, 210 U.S.P.Q. at 692; *see also In re Brebner*, 455 F.2d 1402, 173 U.S.P.Q. 169 (C.C.P.A. 1972) (finding a disclosure enabling because the procedure for making the starting material, although not disclosed, would have been known to one of ordinary skill in the art as evidenced by a Canadian patent). "'That which is common and well known is as if it were written out in the patent and delineated in the drawings.'" *Howarth*, 654 F.2d at 106, 210 U.S.P.Q. at 692 (quoting *Webster Loom Co. v. Higgins* et al., 105 U.S. (15 Otto.) 580, 586 (1881)). Moreover, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed starting materials.

The structure of clostridial neurotoxins was well known prior to the present invention. *See*, for example, the paragraph bridging pages 2 and 3 of the specification. *See also*, the enclosed paper by Kurazono *et al., J. Biol. Chem. 267:*14721-14729) (1992) (hereinafter "Kurazono").

In particular, Kurazono describes the minimum Domains required for proteolytic enzyme activity of a clostridial neurotoxin L-chain. Thus, prior to the present invention, the term "a fragment [of a clostridial neurotoxin L-chain] which includes the active proteolytic enzyme domain of the L-chain" was perfectly well understood by a person of ordinary skill in the art. As such, a person of ordinary skill would not require any further direction from the present patent specification.

By way of exemplification, Table II on page 14726 of Kurazono illustrates a number of L-chain deletion mutants (both amino-terminal and carboxy-terminal L-chain deletion mutants are illustrated). Such mutants, together with other L-chain mutants containing, for example, similar amino acid deletions or conservative amino acid substitutions would fall within the present invention provided that the L-chain or L-chain "fragment" in question possessed the requisite active proteolytic enzyme Domain.

Prior to the present invention a person of ordinary skill could have readily determined whether or not a given L-chain "fragment" possessed the recited active proteolytic enzyme Domain. Thus, no further direction from the present specification is necessary.

In this respect, the L-chain active proteolytic enzyme Domain is associated with peptide cleavage of specific protein components, i.e. synaptobrevin, syntaxin, or

SNAP-25. *See*, the sentence bridging pages 2 and 3 of the specification, and the subsequent part of the same paragraph.

Thus, prior to the present invention, it would have been routine for a person of ordinary skill in the art to test whether a given L-chain "fragment" had the requisite protease activity, e.g. by testing for a cleaved product of the proteolytic reaction. Alternatively, a given L-chain "fragment" could be tested via incorporation into an "agent" of the invention as outlined in any of Examples 4-8 of the present application.

Examples and Further Details of the "Fragment" Enzyme Activity Tests

Methods for detecting an L-chain (or fragment thereof) capable of the requisite protease activity of claim 1 were available prior to the present invention. Thus, a person of ordinary skill would be able to readily determine which L-chain fragments would fall within the scope of claim 1.

For example, in a rough-and-ready test, SNAP-25 (or synaptobrevin, or syntaxin) may be simply challenged with a test L-chain or fragment thereof, and then analyzed by SDS-PAGE peptide separation techniques. Subsequent detection of peptides (e.g. by silver staining) having molecular weights corresponding to the cleaved products of SNAP-25 (or other component of the neurosecretory machinery) would indicate the presence of an L-chain (or a fragment thereof) possessing the requisite protease activity, and thus the active L-chain Domain recited in claim 1.

Alternatively, the test L-chain or fragment thereof may be incorporated into an agent according to Examples 1-3 of the present specification. Thereafter, the agent would

be tested by either *in vitro* challenge (see Examples 4-6 of the present application) or *in vivo* challenge in a mouse experiment (see Examples 7-8 of the present application).

Alternatively, L-chain or fragment cleavage products may be identified by use of the antibodies described in PCT/GB95/01279 (WO 95/33850). In more detail, a specific antibody is employed for detecting cleavage of SNAP-25. Since the antibody recognizes cleaved SNAP-25, but not uncleaved SNAP-25, identification of the cleaved product by the antibody confirms the presence of an L-chain (or a fragment thereof) possessing the requisite active proteolytic Domain. By way of exemplification, such a method is described in Examples 2 and 3 of Foster *et al.*, PCT/GB96/00916 (WO 96/33273), cited on page 9 of the present Office Action).

Summary of the Invention

An agent of the present invention comprises the following three components:

(a) a galactose-binding protein;

(b) a clostridial neurotoxin L-chain including the active proteolytic enzyme Domain of the L-chain (or a fragment thereof including said Domain); and

(c) a molecule or Domain with translocating activity.

The basic methodology provided in Example 1 of the present specification would enable a person of ordinary skill to prepare "agents" across the scope of claim 1 as described below. In this respect, all of the described techniques were part of the state of the art prior to the present invention, and would have been immediately apparent to a person of ordinary skill in the art.

Preparation of an "Agent" of the Present Invention

Example 1 describes how to prepare component (a), in this case a lectin from *E. cristagalli*, for subsequent linking to components (b) and (c) by chemical derivatization. *See*, page 13, top two paragraphs, of the specification.

In Example 1, components (b) and (c) were prepared as a pre-formed conjugate according to the prior art method detailed by Shone *et al.* (1987). *See*, page 12, lines 12-14, of the specification; and then chemically derivatized for subsequent linking to component (a). *See*, page 13, third paragraph, of the specification.

The two derivatized components were then mixed together to form an "agent" in which a galactose-binding lectin, a clostridial neurotoxin L-chain, and a translocating molecule (in this case, the H_N portion of a clostridial neurotoxin H-chain) are linked together. *See*, page 13, fifth paragraph, of the specification.

The above conjugation technique may be readily applied to any embodiment of component (a), (b), and/or (c).

For example, Examples 2 and 3 of the present specification describe how to incorporate different types of component (a), namely different types of galactose-binding lectin.

Similarly, a person of ordinary skill would consider it routine to prepare different component (b) and component (c) combinations.

In more detail, the method of Example 1 follows Shone (1987) and provides a pre-formed conjugate of components (b) and (c). However, prior to the present invention, it was also routine to *separately* prepare components (b) and (c) and to link these

components together by conventional chemical conjugation technology, such as by use of the derivatizing reagent N-succinimidyl 3-(2-pyridyldithio) propionate (i.e. SPDP). This conjugation reagent and the use thereof is described in Example 1 of the present specification.

The separate preparation of components (b) and (c) and subsequent conjugation thereof was routine prior to the present invention as evidenced by Example 6 in PCT/GB94/00558 (WO 94/21300). This Example also employs the conjugation agent SPDP. Alternatively, a different conjugation system (i.e. PDPH/EDAC) is illustrated by Example 4 in PCT/GB96/00916 (WO 96/33273).

Referring to the sentence bridging pages 3 and 4 of the Office Action, the Examiner has referred to "agents" comprising a "fragment of a membrane translocation domain of H-chain from a clostridial toxin." The Examiner would appear to have misunderstood the claim wording, as when the translocation function is provided by a clostridial H-chain, the whole Domain capable of providing this function must be present. Thus, the term "fragment" is not relevant to the translocating component of the claimed agent.

Referring to the same section in the Office Action, the Examiner has commented that the specification does not explicitly illustrate a conjugate containing an L-chain "fragment." However, in view of the above comments, a person of ordinary skill in the art would have no doubt as to the scope of this term, or how to prepare an "agent" comprising such a "fragment." In this respect, a person of ordinary skill would treat an L-chain "fragment" in exactly the same manner as he would an entire L-chain, and the conjugation steps may be performed in an identical manner.

In the same section bridging pages 3 and 4 of the Office Action, the Examiner has alleged that the specification does not enable preparation of an "agent" by chemical or recombinant techniques. However, Example 1 explicitly describes preparation of an "agent" by chemical conjugation.

Regarding recombinant preparation of a claimed "agent," page 10, top two paragraphs, of the specification provides general guidance, and suitable recombinant techniques were well known prior to the present invention to allow a person of ordinary skill to prepare the presently claimed "agents." This is evidenced by Kurazono which describes the recombinant preparation of various L-chains (*see*, for example, the "Materials and Methods" on page 14722), and by the following additional evidence, which support claim 50.

Additional Evidence in Support of Claim 50

A genetic construct may be employed which encodes a galactose-binding lectin, the L-chain of a clostridial neurotoxin (in this case, the entire L-chain), and a translocating Domain (in this case, the H_N portion of a clostridial neurotoxin).

The coding sequences of the galactose-binding lectin and clostridial neurotoxin components are arranged in a single genetic construct. These coding sequences are arranged in-frame so that subsequent transcription and translation is continuous through both coding sequences and results in a fusion protein. The construct has a 5' ATG codon to encode an N-terminal methionine, and a C-terminal translational stop codon.

The method of claim 50 requires nucleic acid sequence data encoding the selected galactose-binding lectin and the clostridial neurotoxin components. These DNA sequence data were readily available at the priority date of the present application.

Obtaining the Necessary DNA Sequence Data for the Galactose-binding Lectin Component of the Construct in Claim 50

Several galactose-binding lectins are listed at page 8, line 16, through page 9, line 18, of the specification, and below, which confirms that the necessary nucleic acid sequence data were available for galactose-binding lectins prior to the priority date (i.e. October 8, 1997) of the present application:

The following galactose-binding lectin nucleic acid sequences are illustrative of those which were readily available by the priority date of the present application (i.e. October 8, 1997):

1) EcorL lectin

Arango *et al., FEBS Lett. 264*:109-111 (1990) ;

2) Abrin lectin

Wood *et al., Eur. J. Biochem. 198*:723-732 (1991) ;

3) Ricin lectin

Lamb *et al., Eur. J. Biochem. 148*:265-270 (1985) ;

4) Peanut nodule lectin

Law, I. J. *et al., Plant Sci. 115*:71-79 (1996) ; and

5) Sophora japonica lectin

Van Damme, E. J. *et al., Plant Mol. Biol. 33*:523-536 (1997) .

As detailed above, suitable nucleic acid data could also have been readily obtained by, for example, back-translation from corresponding amino acid sequence data.

The following Example describes how to make an *E. cristagalli* (ExL) lectin-LH_N/A fusion construct in accordance with Claim 50. ExL lectin has been selected for the purpose of this illustration simply because conjugates comprising this lectin Targeting Moiety are preferred aspects of the present invention, and chemical conjugation methods for preparing such conjugates have already been described in the specification of the present application (see Example 1). However, any of the above-identified lectin DNA sequences would be equally suitable for the purpose of the present recombinant method illustration.

The coding region for ExL lectin is placed in frame with the coding sequence for LH_N/A such that translation of protein is continuous through the ExL lectin and into LH_N/A coding sequence. Alternatively, the LH_N/A coding sequence may be placed upstream of the ExL lectin coding sequence such that translation of protein is continuous through the LH_N sequence and into the ExL coding sequence.

Translation is initiated by incorporation of the codon for an N-terminal methionine (ATG) immediately before the ExL lectin sequence.

Translation is terminated by incorporation of a STOP codon (TGA, TAA or TAG) immediately after the LH_N/A coding sequence, unless it is preferred to use a C-terminal tag to facilitate purification of the fusion protein, in which case no STOP codon would be inserted and translation would continue into the tag.

The entire DNA expression cassette is then cloned into, for example, a vector suitable for expression of proteins in *E. coli*. Expression of the protein is achieved by

induction of transcription and the synthesized protein is isolated from the host cell by classical purification techniques. Incorporation of an affinity tag would facilitate this last step.

Alternatively, any necessary galactose-binding lectin sequence data may be obtained by techniques which were well known to the person of ordinary skill prior to the present application. In this respect, it was routine prior to the present application to generate DNA sequence data from existing protein and/or RNA sequence information. If desirable, optimization of the codon bias is possible by application of the protein sequence into freely available DNA/protein database software, e.g. programs available from Genetics Computer Group, Inc.

By way of example, suitable amino acid data were readily available for galactose-binding lectin derived from *Erythrina variegata*. *See*, Yamaguchi *et al.*, *J. Biochem., Tokyo, 114*:560-566 (1993), and EcorL lectin which is compared with nine other legume lectin amino acid sequences in *Adar et al., FEBS Lett. 257*:81-85 (1989).

Alternative routine methods are briefly described below:

(a) DNA encoding the galactose-binding lectin component may be cloned from a source organism by screening a cDNA library for the correct coding region (for example by using specific oligonucleotides based on the known sequence information to probe the library), isolating the galactose-binding lectin DNA, sequencing this DNA for confirmation purposes, and then placing the isolated DNA in an appropriate expression vector for expression in the chosen host; or

(b) Available sequence information may be employed to prepare specific primers for use in PCR, whereby the coding sequence is then amplified directly from the source

material and, by suitable use of primers, may be cloned directly into an expression vector.

Obtaining the Necessary DNA Sequence Data for the Clostridial Components of the Construct in Claim 50

Clostridial neurotoxins comprise a heavy chain (H-chain) and a light chain (L-chain). For the purpose of repeatability of claim 50, all that would be required is:

(i) DNA sequence data for the L-chain; and

(ii) DNA sequence data for a Translocation Domain, such as the H_N component of the clostridial H-chain.

In this respect, suitable sequence data for the LH_N of a clostridial neurotoxin were readily available prior to the present application:

by way of example, Fig. 3 on page 14724 of Kurazono confirms that amino acid sequence data for L-chains were readily available at the priority date of the present invention.

Other publications (not enclosed) exist to support the availability of L-chain and H_N component DNA sequences prior to the present application, such as:

Binz *et al., J. Biol. Chem. 265*:9153-9158 (1990) ;

Niemann in "Sourcebook of bacterial protein toxins." (J. E. Alouf & J. H. Freer, eds.), Academic Press, London, United Kingdom, pp. 303-348 (1991); and

Henderson *et al.* "The genetic basis of toxin production in *Clostridium botulinum* and *Clostridium tetani*." Chapter 17 in the *Clostridia*: Molecular Biology and Pathogenesis, Academic Press Ltd., published in April/May of 1997.

Furthermore, all of the described methods for identifying relevant DNA sequence data for galactose-binding lectins may be employed to obtain DNA sequence data on the selected clostridial neurotoxin component of the construct of claim 50.

Construction and Expression of a Construct According to Claim 50

Expression of fusion proteins was well known at the priority date of the present application (i.e. October 8, 1997). Methods for the construction and expression of the constructs of the present invention may employ information from the following references and others:

Lorberboum-Galski, H. *et al.*, "Cytotoxic activity of an interleukin 2-Pseudomonas exotoxin chimeric protein produced in Escherichia coli," *Proc. Natl. Acad. Sci. USA 85*:1922-1926 (1988);

Murphy, J.R. "Diphtheria-related peptide hormone gene fusions: a molecular genetic approach to chimeric toxin development," *Cancer Treat Res. 37*:123-140 (1988);

Williams, D.P., *et al.*, "Diphtheria toxin receptor binding domain substitution with interleukin-2: genetic construction and properties of a diphtheria toxin-related interleukin-2 fusion protein," *Protein Eng. 1*:493-498 (1987);

Arora, N. *et al.*, "Cytotoxic effects of a chimeric protein consisting of tetanus toxin light chain and anthrax toxin lethal factor in non-neuronal cells," *J. Biol. Chem. 269*:26165-26171 (1994);

Brinkmann, U. *et al.*, "A recombinant immunotoxin containing a disulphide-stabilized Fv fragment," *Proc. Natl. Acad. Sci. USA 90*:7538-7542 (1993); and

O'Hare, M. *et al.*, "Cytotoxicity of a recombinant ricin-A-chain fusion protein containing a proteolytically-cleavable spacer sequence," *FEBS Lett. 273*:200-204 (October 29, 1990).

Thus, all that a person of ordinary skill would require in order to prepare a recombinant "agent" of the present invention would be the relevant nucleic acid information relating to the particular "agent" which is to be prepared.

As demonstrated above, all requisite information was readily available prior to the present invention. Thus, a person of ordinary skill would require no further direction from the present specification in order to recombinantly prepare an "agent" of the present invention.

In the sentence bridging pages 3 and 4, the Examiner has also alleged that the present specification does not illustrate use of an "agent" for controlling the sensation of pain. However, current Example 8 explicitly illustrates such use. In more detail, an "agent" of the present invention is administered intrathecally to a mouse, and the mouse then placed on a hot metal plate. The mouse stands on the plate until it senses pain. Figure 10 illustrates the superior analgesic effect of an "agent" of the present invention *vis-a-vis* a conventional analgesic (i.e. morphine).

It would be unnecessary for a person of ordinary skill to be presented with any further information by the present specification in order to administer further embodiments of the claimed "agent." In view of this, it is unreasonable to require an

Example illustrating administration of each embodiment falling within the scope of claim 1.

Starting at page 4, line 4, of the Office Action, the Examiner has commented that the specification provides no exemplification or data for an "agent" comprising a lectin which has been recombinantly produced. This is evidenced by the enclosed Abstract by Arango (1992) which describes the recombinant preparation of ECorL lectin from *Erythrina corallodendron*. The recombinant preparation of an "agent" of the present invention is described above, and it is clear that the recombinant preparation of a lectin would have been routine prior to the present application. In any case, for expediency, claim 17 has been canceled.

In the same paragraph, the Examiner has alleged that the specification provides no exemplification of modifying a lectin enzymatically or chemically. Surely the Examiner does not believe that a person of ordinary skill in this art would require exemplification in order to be able to contact a lectin with a chemical or enzyme. Claims 18 and 19 have now been amended to delete the word "modified" and to qualify the modification such that a modified lectin must still possess the defined binding ability which is required by "agents" of the present invention.

Evidence of Analgesic Effects of the "Agents" of the Present Invention

Starting at page 4, line 7, of the Office Action, the Examiner has alleged that, based on the experimental data presented in the specification, it would be "hard to predict" whether a given conjugate would be effective, even *in vitro*. In order to make a

rejection for lack of enablement, it is the burden of the Examiner to establish a reasonable basis to question the enablement provided for the claimed invention. Applicants submit that the Examiner has not met this burden.

All that is required in order to enable the present invention is a description of how to make and use the claimed "agent." In this respect, Examples 1-3 describe how to make the claimed "agent," and current Examples 4-7 (*see* also page 11, lines 5-10) describe how to test and/or administer the claimed "agent."

Thus, the present specification describes how to make, how to test the effectiveness of, and how to use the claimed "agent," and is therefore "enabled" in the context of 35 U.S.C. § 112.

It is not necessary to exemplify the preparation or to illustrate the effectiveness of each and every embodiment falling within the scope of a claim. The type of data which the Examiner has referred to on page 5 of the Office Action are the type of data which might be associated with a clinical trial. Such a level of data is not required to support "enablement" of a patent application.

A person of ordinary skill does not need to be explicitly taught how to employ alternative, *conventional* techniques for making a claimed "agent," how to employ *readily interchangeable* components of the claimed "agent" (i.e. components (a)-(c) above), or how to employ *conventional* routes of administering an "agent" to a patient.

At the bottom of page 4 of the Office Action, the Examiner has referred to a publication by Welch (2000). In particular, the Examiner has cited Welch to support an assertion that, because Welch determined an IC_{50} difference of 1000-fold between the A and B serotype *C. botulinum* neurotoxins, there should be doubts that "agents" of the

present invention comprising L-chain components from different neurotoxin serotypes would exert an analgesic effect. This conclusion is not correct for two reasons.

First, the Welch article describes use of clostridial *holotoxin* which would, of course, contain the native Targeting Moiety (TM) of holotoxin (i.e. H_C) for motor neurons. In contrast, the "agents" of the present invention contain a galactose-binding lectin as a TM. As a result of the different TMs employed, the processes involved in binding to a receptor on the DRG target cell, and possibly the subsequent translocation step, would be different for holotoxin and for the "agents" of the present invention. Thus, a given ID_{50} value obtained with a particular holotoxin serotype is not relevant to a corresponding ID_{50} value for an "agent" of the present invention employing the same L-chain serotype.

Secondly, just because a particular *C. botulinum* serotype neurotoxin demonstrates a 1000-fold lower IC_{50} activity than that of a different serotype, it should be appreciated that the lower activity may be perfectly adequate to achieve a desired clinical effect (i.e. in the context of the present invention, a desired analgesic effect). In this respect, the enclosed "NeuroBloc" publication makes it perfectly clear that BoNT serotype B neurotoxin is suitable for providing a desirable *in vivo* effect. In more detail, NeuroBloc is a commercial product based on BoNT serotype B holotoxin for treating cervical dystonia.

Towards the bottom of page 4 of the Office Action, the Examiner has made a similar objection with respect to "agents" of the present invention which possess different types of galactose-binding lectin. However, again the Examiner's conclusion is incorrect.

In particular, the Examiner does not appear to have appreciated that one of the important data comparisons which is relevant to assessing the analgesic effectiveness of an "agent" of the present invention is a comparison of the IC_{50} values for eDRG and eSC. In particular, a low IC_{50} value alone does not necessarily indicate a preferred analgesic agent. For example, if a conjugate were to demonstrate a low IC_{50} value for both eDRG and eSC, then such a conjugate would lack a desired selectivity for pain-sensing cells.

Thus, an "agent" of the present invention should preferably demonstrate a low value of:

$$\frac{IC_{50} \text{ for eDRG}}{IC_{50} \text{ for eSC}} .$$

Referring to the Examples and Figures of the present specification, it is clear that all of the illustrated "agents" demonstrate a significantly superior ratio value (based on the above formula) than do other structurally related conjugates which contain a lectin other than a galactose-binding lectin.

In particular, the ExL-based "agent" of Example 4 demonstrates an eDRG value of 3.66 (see page 20, line 21, of the specification). Referring to Fig.6, the extrapolated corresponding eSC value would be significantly in excess of 100. Thus, the ratio for this "agent" would be significantly less than 0.03.

Similarly, the SBA-based "agent" of Example 5 demonstrates an eDRG value of about 4.7 (i.e. an average of the 2 values at page 21, line 29), and an extrapolation of Fig.7 provides a corresponding eSC value in excess of 100. Thus, the ratio for this "agent" would be less than 0.04.

However, when the eDRG and eSC values are compared for the non-galactose-binding lectin conjugate of Example 6 (*see* page 22, lines 19-22), a ratio value of 5.67 is obtained.

Thus, even the SBA-based "agent" of the present invention demonstrates an approximate 100-fold superior ID_{50} ratio than does the WGA-based (i.e. non-galactose-binding lectin) conjugate of Example 6. Accordingly, the Examiner's conclusion (see the bottom of page 4 of the Office Action) with respect to the effectiveness of "agents" of the present invention comprising different types of galactose-binding lectin is incorrect.

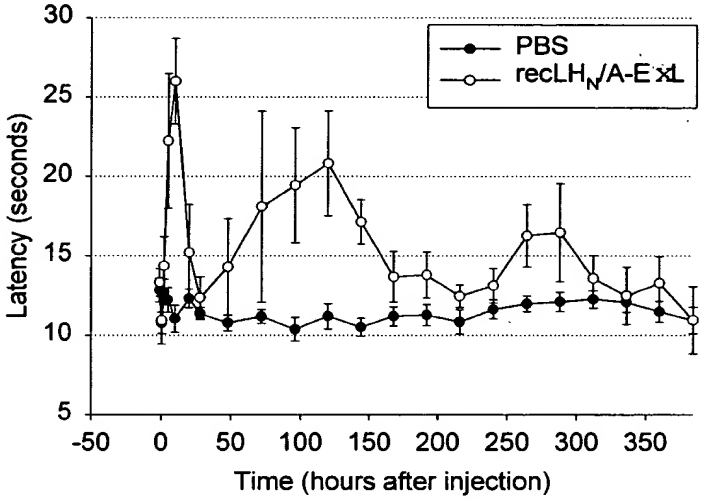
In summary, the specification describes how to prepare a claimed "agent" (*see* Examples 1-3), and how to test and/or administer said "agent" (*see* current Examples 4-7, and page 11, lines 5-10). Thus, contrary to the Examiner's allegations, a person of ordinary skill would not require undue experimentation in order to make and use "agents" across the scope of the present claims.

Additional Experimental Data

Additional data is presented below to provide further support for the utility of the "agents" of the present invention.

Data obtained for conjugate of recLH$_N$/A-ExL (where ExL is derived from a plant source).

Figure 1. Comparison of effect of morphine (1 μg), vehicle (phosphate buffered saline) and a single dose of *rec*LH_N/A-ExL (25 μg) in the mouse hotplate model. Hotplate temperature = 55°C. Data obtained from the same study, but morphine data plotted on expanded axes for clarity. Note the extended duration of action of the *rec*LH_N/A-ExL conjugate



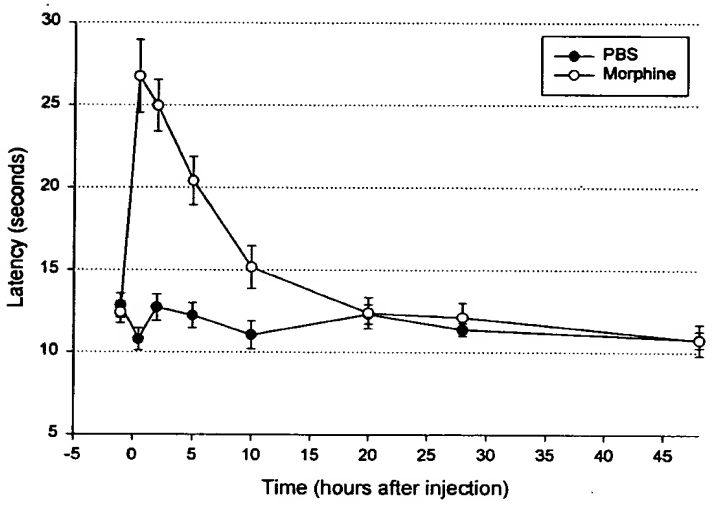
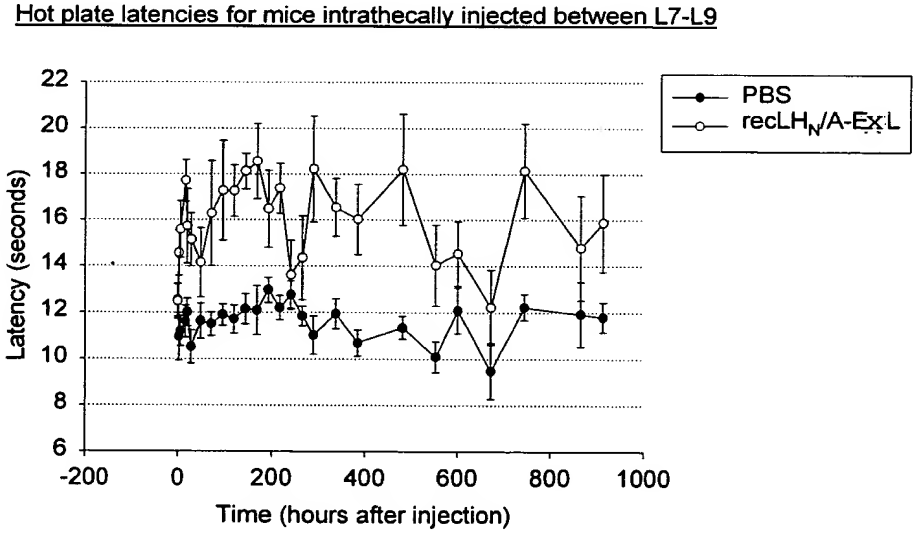
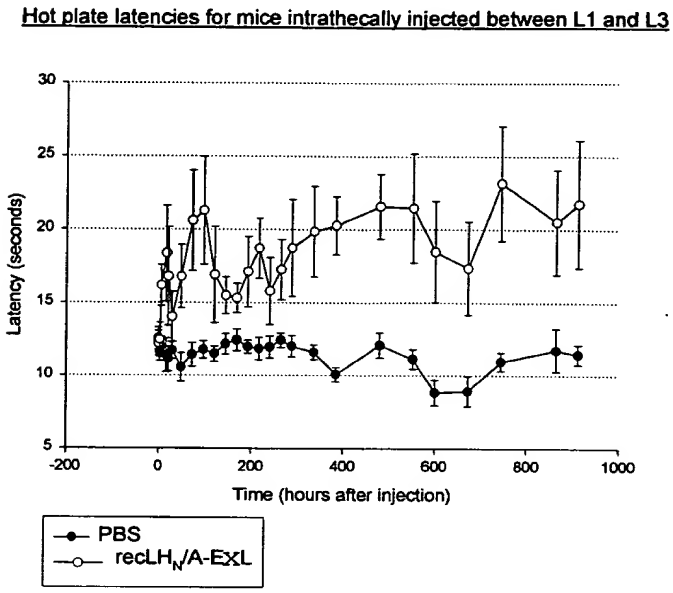


Figure 2. Comparison of effect of a single dose of *rec*LH_N/A-ExL (25 μg) and vehicle (phosphate buffered saline) in the mouse hotplate model. Hotplate temperature = 55°C. Data obtained following intrathecal application of test material at two sites in the mouse spinal cord. Note the extended duration of action of the *rec*LH_N/A-ExL conjugate





These data relate to an "agent" which has been prepared by chemical conjugation of a galactose-binding lectin (i.e. ExL) to a recombinantly produced clostridial component comprising the proteolytically active Domain of the L-chain and a translocation Domain (i.e. LH_N/A).

The above "agent" was then tested in a mouse "hot plate" test. The "hot plate" test is a conventional and widely accepted test for assessing the effectiveness of an analgesic molecule.

In Figure 1, above, the analgesic effectiveness of an "agent" of the present invention has been compared with that of a conventional analgesic, morphine. The top Figure illustrates that the claimed "agent" provides an analgesic effect *vis-a-vis* phosphate buffer saline (PBS) for test periods of 50 hours and more post-injection. In contrast, no such analgesic effect is observed with morphine for test periods of 20 hours and more post-injection.

Figure 2, above, illustrates the analgesic effect of the same "agent" when injected at different vertebrae sites in a test mouse. This Figure confirms good analgesic activity even after 900 hours post-injection.

In view of the remarks above, Applicants submit that the rejection has been overcome. Withdrawal of the rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-54 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants respectfully traverse this rejection.

The Examiner states:

> The terms "a derivative of a clostridial neurotoxin" and "the L-chain, or a fragment" render the claim indefinite, it is unclear in the claim what kind of peptide is obtained as a derivative of clostridial neurotoxin as compared to the parent compound, and what kind of peptide is intended for the fragment of L-chain containing the active proteolytic enzyme domain. Is the derivative obtained from the neurotoxin or is it a modified form? Claims 2-54 are included in the rejection for being dependent of a rejected claim and not correcting the deficiency of the claim from which they depend.
>
> Claim 1 is also indefinite regarding "an agent for the treatment of pain that comprises" or is it "the treatment of pain that comprises"? Two commas, one after "agent" and one after "pain" would appear to be necessary.

(Office Action, page 6, lines 11-21).

Claim 1 has been amended to delete the term "derivative." In addition, commas have been inserted after "agent" and before "pain" as requested by the Examiner.

The term "L-chain" is a recognized term in the art, as evidenced by Fig. 1 on page 14722 of Kurazono.

The term "fragment" has been clarified by the presence of "the active proteolytic enzyme domain of the L-chain." This functional requirement has been discussed in detail above.

Claim 1 has been revised as requested by the Examiner to make it clear that it is the noun (i.e. the "agent" per se) which comprises the defined structural features, rather than the intended use of the "agent."

Claim 2 is considered by the Examiner to be indefinite because of the use of the term "is derived from." The Examiner states:

> The term "is derived from" renders the claim indefinite, it is unclear in the claim what kind of peptide is obtained from the heavy chain of clostridial toxin for the translocation domain. The same rejection is also applied to claims 3, 7-10, 42, 48 and 49. Use of the term "is obtained from" is suggested.

(Office Action, bridging pages 6 and 7).

The term "derived from" has been deleted from claim 2, and revised to "obtained from" in the subsequent claims as suggested by the Examiner.

The Examiner considers claims 3 and 49 indefinite because of the use of the term "non-clostridial source." The Examiner states that "[t]he term 'non-clostridial source' renders the claim indefinite, it is unclear in the claim what the non-clostridial source is." (Office Action, page 7, lines 5-6).

The Examiner also states that "[c]laim 3 recites the limitation 'non-clostridial source' in line 2. There is insufficient antecedent basis for this limitation in the claim 1. The same rejection is also applied to claim 49." (Office Action, page 7, lines 7-8).

The term "non-clostridial source" has been deleted from claims 3 and 49, and the various preferred, non-clostridial sources listed from page 10, last paragraph, of the specification, has been added in place thereof.

The Examiner states that "[c]laim 17 is indefinite as to how 'recombinant technology' makes the claimed product differ from non-recombinant technology." (Office Action, page 7, lines 9-10).

For the purpose of expediting prosecution claim 17 has been deleted.

The Examiner states:

> Claims 18-23, 42 and 43 are indefinite because of the use of the term "enzymatically modified", "chemically modified", "the Hc domain of H-chain is removed or modified", "modified by chemical derivatisation", "modified by mutation", "modified by proteolysis", "protein modification", "lectin protein has been modified" or "modification of the nucleic acid". The term "enzymatically modified", "chemically modified", "the Hc domain of H-chain is removed or modified", "modified by chemical derivatisation", "modified by mutation", "modified by proteolysis", "protein modification", "lectin protein has been modified" or "modification of the nucleic acid" renders the claim indefinite, it is unclear in the claim what kind of modification is performed on the peptide or nucleic acid and where the modification occurs.

(Office Action, page 7, lines 11-20).

Claims 18 and 19 have been amended to remove the term "modified," and to clarify the result of the modification, namely retention of an ability to bind to galactose or N-acetylgalactosamine (see claim 42).

Claim 20 has been amended to clarify the purpose and result of the removal/modification of the H_C Domain, namely to remove/reduce the native binding capacity of the "agent" for motor neurons. Moreover, the term "H_C Domain" is a recognized term in the art, as evidenced by Fig. 1 on page 14722 of Kurazono, and the phrase "the H_C part [Domain] of the H-chain is removed or modified" is perfectly clear.

Claims 21-23 have now been made dependent on claim 20 and the term "modified" has been deleted.

In claim 22, the various types of mutation have been listed exhaustively.

Claims 42 and 43 have been amended by deletion of the term "modified" or "modification," and by exhaustively listing the various types of mutation.

The Examiner states:

> Claims 20-24 and 32-37 are indefinite because of the use of the term "if the heavy chain (H-chain) of a clostridial neurotoxin is present" or "the H-chain, if present". The term "if the heavy chain (H-chain) of a clostridial neurotoxin is present" or the "the H-chain, if present" renders the claim indefinite, it is unclear as to what happens when the H-chain is not present, e.g., can it have a H_C domain? Or can it have H_N fragment? Claims 33-35 are included in the rejection for being dependent of a rejected claim and not correcting the deficiency of the claim from which they depend.

(Office Action, bridging pages 7 and 8).

The term "if the H-chain is present" (or equivalent wording) has been deleted, and the relevant claims have been made dependent (either directly or indirectly) on claim 2 which recites the presence of the H-chain.

Claims 37, 40 and 47 were rejected as indefinite for recitation of the term "one or more spacer regions." The Examiner indicates that "it is unclear in the claim how many spacer regions are included." (Office Action, page 8, lines 7-8).

The term "one or more" has been replaced in claims 37, 40 and 47 by the wording "at least one." Applicants submit that this phase is clear.

Claims 39 and 40 were rejected as indefinite for use of the term "clostridial neurotoxin-derived component." The Examiner states:

> The term "clostridial neurotoxin-derived component" renders the claim indefinite, it is unclear in the claim which component is from clostridial neurotoxin and what kind of peptide is obtained as compared to the parent compound.

(Office Action, page 8, lines 10-13).

The term "clostridial neurotoxin-derived component" has been deleted from claims 39 and 40.

Claims 51-54 were rejected as indefinite. The Examiner states:

> Claims 51-54 are indefinite because they lack essential steps as claimed in the process of controlling the transmission of sensory information. The omitted steps are: the site and method for applying the agent, the effective amount of the agent and a step whereby the desired outcome can be determined.

(Office Action, page 8, lines 13-16).

A claim does not lack clarity simply because a particular route of admission has not been recited. The phrase "an effective amount" has been added to each of claims 51, 52 and 54. New claims 58-62 have been added in which preferred modes of administration have been defined (*see* page 11, lines 2-4, of the specification).

In view of the amendments and remarks above, Applicants request that the Examiner withdraw the rejection under 35 U.S.C. § 112, second paragraph.

Rejections Under 35 U.S.C. § 102

Claims 1, 2, 4-48 and 50-54 were rejected under 35 U.S.C. § 102 as being anticipated by Foster *et al.* (WO 96/33273) (hereinafter "Foster").

The Examiner states:

> Foster *et al.* teach an agent containing the lectin (page 13, lines 9-13) as the TM component and a clostridial neurotoxin such as modified L chain, modified H_N and H_C of heavy chain, and the corresponding fragment (page 13, line 18-page 14, line 19) can be obtained by covalently attachment of a TM to a modified clostridial neurotoxin using linkage including one or more spacer regions (page 14, lines 1-9) or can be expressed recombinantly as a fusion protein (page 14, line 29-page 15, line 4), which meet the criteria of claims 1-2, 4-48 and 50. This agent can bind to a binding site on the surface of sensory neurons (page 12, lines 25-28) and reduce and preferably prevent the transmission of pain signals from nociceptive afferents to projection neurons (page 7, lines 15-17), therefore it can be used for controlling the transmission of sensory information or pain signals from a nociceptive afferent to a projection neuron, which meets the criteria of claims 51-54.

(Office Action, page 9, lines 3-13).

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. MPEP § 2131. Applicants submit that Forster *et al.* do not teach each and every element of claims 1, 2, 4-48 and 50-54. Therefore, the rejection is in error and must be withdrawn.

The present invention is directed to a *new class of analgesic toxin conjugate* comprising a specific Targeting Moiety (i.e. a galactose-binding lectin).

This Targeting Moiety is not described anywhere by Foster. Withdrawal of the rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

Robert W. Esmond
Attorney for Applicants
Registration No. 32,893

Date: Dec. 14, 2001

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

P107-09.wpd

Version with markings to show changes made

In the Specification:

At page 22, lines 1-2, the heading has been amended as follows:

Example [7]6. Activity of WGA-LH_N/A in primary neuronal cultures

At page 22, lines 23-24, the heading has been amended as follows:

Example [8]7. Activity of ExL-LH_N/A in an electrophysiological model of pain

At page 23, lines 12-13, the heading has been amended as follows:

Example [9]8. Activity of ExL-LH_N/A in behavioural models of pain

In the Claims:

Claim 17 has been canceled.

Claims 1-13, 15, 16, 18-34, 36–43, 46-49, 51, 52 and 54 have been amended as follows:

1. (Once amended) An agent, for the treatment of pain, that comprises:- a galactose-binding lectin; [linked to a derivative of a clostridial neurotoxin, in which the derivative of the clostridial neurotoxin comprises the L-chain, or] a light (L) chain or an L-chain fragment of a clostridial neurotoxin [thereof], which L-chain or L-chain fragment includes the active proteolytic enzyme domain of the [light (L)] L-chain; [, linked to] and a molecule or domain with membrane translocating activity; wherein the galactose-binding lectin, L-chain or fragment, and molecule or domain with membrane translocating activity are linked together.

2. (Once amended) An agent according to Claim 1 in which the membrane translocation domain is [derived from the] a heavy (H) chain of a clostridial toxin.

3. (Once amended) An agent according to Claim 1 in which the membrane translocation domain is [derived from a non-clostridial source] selected from the group consisting of a translocation domain of diphtheria toxin, a translocation domain of pseudomonas exotoxin, a translocation domain of anthrax toxin, and a translocating fusogenic peptide.

4. (Twice amended) An agent according to Claim 1 in which the lectin binds to an oligosaccharide[s] that contains an exposed [terminal] β-D-galactosyl residue[s].

5. (Twice amended) An agent according to Claim 1 in which the lectin binds to an oligosaccharide[s] that contains an exposed [terminal] α-D-galactosyl residue[s].

6. (Twice amended) An agent according to Claim 1 in which the lectin binds to an oligosaccharide[s] that contains an exposed N-acetylgalactosamine residue.

7. (Twice amended) An agent according to Claim 1 in which the lectin [is derived] has been obtained from a species of plant.

8. (Twice amended) An agent according to Claim 1 in which the lectin [is derived] has been obtained from a species of the genus *Erythrina*.

9. (Once amended) An agent according to Claim 8 in which the lectin [is derived] has been obtained from *E. cristagalli*.

10. (Once amended) An agent according to Claim 8 in which the lectin [is derived] has been obtained from *E. corallodendron*.

11. (Twice amended) An agent according to Claim 7 in which the lectin [is] has been obtained from *Glycine max*.

12. (Twice amended) An agent according to Claim 7 in which the lectin [is] has been obtained from *Arachis hypogaea*.

13. (Twice amended) An agent according to Claim 7 in which the lectin [is] has been obtained from *Bandeirea simplicifolia*.

15. (Twice amended) An agent according to Claim 1 in which the lectin is [obtained from bacteria] of bacterial origin.

16. (Once amended) An agent according to Claim 15 in which the lectin [is] has been obtained from *Pseudomonas aeruginosa*.

18. (Twice amended) An agent according to Claim 1 in which the lectin has been [enzymatically modified] contacted with an enzyme, and retains an ability to bind to an oligosaccharide structure having an exposed galactose or N-acetylgalactosamine residue.

19. (Twice amended) An agent according to Claim 1 in which the lectin has been [chemically modified] contacted with a chemical, and retains an ability to bind to an oligosaccharide structure having an exposed galactose or N-acetylgalactosamine residue.

20. (Twice amended) An agent according to Claim [1 wherein, if the heavy chain (H-chain) of a clostridial neurotoxin is present,] 2, wherein the H_C domain of the H-chain [is] has been removed or modified to remove or reduce the native binding affinity of the H-chain for motor neurons.

21. (Twice amended) An agent according to Claim [1 in which the H-chain, if present, is modified by chemical derivatisation] 20, wherein the H-chain has been contacted with a derivatising chemical to reduce or remove [its] the native binding affinity of the H-chain for motor neurons.

22. (Twice amended) An agent according to Claim [1 in which the H-chain, if present, is modified by mutation] 20, wherein the H-chain has been mutated by the inclusion of at least one amino acid deletion, insertion, and/or substitution to reduce or remove [its] the native binding affinity of the H-chain for motor neurons.

23. (Twice amended) An agent according to Claim [1 in which the H-chain, if present, is modified by proteolysis] 20, wherein the H-chain has been contacted with a proteolytic agent to reduce or remove the native binding affinity of the H-chain for motor neurons.

24. (Once amended) An agent according to Claim 20 in which [, if the H-chain is present,] the H_C domain [is] has been completely removed leaving [only] the H_N-fragment of a clostridial neurotoxin.

25. (Twice amended) An agent according to Claim 1 in which the [derivative] L-chain of the clostridial neurotoxin [, or fragment thereof,] is [obtained from] a botulinum neurotoxin L-chain.

26. (Twice amended) An agent according to Claim [1] 25 in which the [derivative] L-chain of the clostridial neurotoxin [, or fragment thereof,] is [obtained from] a botulinum neurotoxin type A L-chain.

27. (Twice amended) An agent according to Claim [1] 25 in which the [derivative] L-chain of the clostridial neurotoxin [, or fragment thereof,] is [obtained from] a botulinum neurotoxin type B L-chain.

28. (Twice amended) An agent according to Claim 1 which [is] has been formed by the coupling of a galactose-binding lectin to the LH_N fragment of botulinum neurotoxin type A.

29. (Once amended) An agent according to Claim 28 which [is] has been formed by the coupling of the galactose-binding lectin from *Erythrina cristagalli* to the LH_N fragment of botulinum neurotoxin type A.

30. (Once amended) An agent according to Claim 28 which [is] has been formed by the coupling of the galactose-binding lectin from *Erythrina corallodendron* to the LH_N fragment of botulinum neurotoxin type A.

31. (Once amended) An agent according to Claim 28 which [is] has been formed by the coupling of the galactose-binding lectin from *Glycine max* to the LH_N fragment of botulinum neurotoxin type A.

32. (Twice amended) An agent according to Claim [1] 2 in which the H-chain [, if present, is] has been obtained from a different clostridial neurotoxin than that from which the L-chain [is] or fragment thereof has been obtained.

33. (Once amended) An agent according to Claim 32 in which the H-chain [is] has been obtained from botulinum neurotoxin type A and the L-chain or fragment thereof from botulinum neurotoxin type B.

34. (Once amended) An agent according to Claim 32 in which the H-chain [is] has been obtained from botulinum neurotoxin type A and the L-chain or fragment thereof from tetanus neurotoxin.

36. (Twice amended) An agent according to Claim [1] 2 in which the L-chain or L-chain fragment is linked to the H-chain [, if present,] by a direct covalent linkage.

37. (Twice amended) An agent according to Claim [1] 2 in which the L-chain or L-chain fragment is linked to the H-chain [, if present,] by a covalent linkage which includes at least one [or more] spacer region[s].

38. (Twice amended) An agent according to Claim 1 in which the [clostridial neurotoxin derivative incorporates] L-chain or fragment is a polypeptide[s] produced by recombinant technology.

39. (Twice amended) An agent according to Claim 1 in which the lectin is linked to the [clostridial neurotoxin-derived component] L-chain or fragment thereof, and/or to the molecule or domain with membrane translocating activity by a direct covalent linkage.

40. (Twice amended) An agent according to Claim 1 in which the lectin is linked to the [clostridial neurotoxin-derived component] L-chain or fragment thereof, and/or to the molecule or domain with membrane translocating activity by a covalent linkage which includes at least one [or more] spacer region[s].

41. (Twice amended) An agent according to Claim 1 in which the lectin [and clostridial neurotoxin components] , L-chain or fragment thereof, and molecule or domain with membrane translocating activity are produced as a recombinant fusion protein.

42. (Twice amended) An agent according to Claim 1 in which the lectin protein has [been modified from its native polypeptide sequence whilst retaining an ability for the protein to bind to oligosaccharide structures, in which the terminal residue is derived from] at least one amino acid insertion, deletion, or substitution when compared with the polypeptide sequence of the corresponding native lectin protein, and retains an ability to bind to an oligosaccharide structure having an exposed galactose or N-acetylgalactosamine residue.

43. (Once amended) An agent according to Claim 42 in which the [protein modification results from modification of the nucleic acid coding for the lectin protein from its native sequence] nucleic acid coding for the lectin protein has at least one nucleotide deletion, insertion and/or substitution when compared with the nucleic acid sequence coding for the corresponding native lectin protein.

46. (Twice amended) A method for obtaining an agent according to Claim 1 which comprises:- the covalent attachment of a galactose-binding lectin [to a derivative of a clostridial neurotoxin, in which the derivative of the clostridial neurotoxin comprises the] , an L-chain or an L-chain fragment of a clostridial neurotoxin which L-chain or an L-chain fragment includes the active proteolytic domain of the [light (L)] L-chain, [linked to] and a molecule or domain with membrane translocating activity ; thereby providing an agent in which the galactose-binding lectin, L-chain or L-chain fragment, and molecule or domain with membrane translocating activity are linked together.

47. (Twice amended) A method for obtaining an agent according to Claim [1 which comprises] 46 wherein the covalent attachment [of a galactose-binding lectin to a derivative of a clostridial neurotoxin with the inclusion of] includes at least one [or more] spacer region [s, in which the derivative of the clostridial neurotoxin comprises the L-chain or an L-chain fragment which includes the active proteolytic enzyme domain of the light (L) chain, linked to a molecule or domain with membrane translocating activity].

48. (Twice amended) A method according to Claim 46 in which the membrane translocation domain is [derived from] the heavy chain of a clostridial toxin.

49. (Twice amended) A method according to Claim 46 in which the membrane translocation domain is [derived from a non-clostridial source] selected from the group consisting a translocation domain of diphtheria toxin, a translocation domain of pseudomonas exotoxin, a translocation domain of anthrax toxin, and a translocating fusogenic peptide.

51. (Twice amended) A method of controlling the transmission of sensory information from a primary sensory afferent to a projection neuron by [applying] administering an effective amount of the agent of Claim 1.

52. (Twice amended) A method of controlling the transmission of sensory information from a primary nociceptive afferent to a projection neuron by [applying] administering an effective amount of the agent of Claim 1.

54. (Twice amended) A method of controlling the sensation of pain by [applying] administering an effective amount of the agent of Claim 1.

New claims 58-62 have been added.